GREEN SYNTHESIS OF SILVER NANOPARTICLES USING GOSSYPIUM ARBOREUM LEAF EXTRACT AND IT'S ANTIMICROBIAL EFFICACY (COTTON LEAF)

PRESENTED BY

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CERTIFICATION

This is to certify that this research project was written by **KAYODE FUNMILAYO RUTH HND/23/SLT/FT/0685** and submitted to the department of Science Laboratory Technology, Microbiology unit, Institute of Applied Science, Kwara State Polytechnic, Ilorin, and has been read and approved as partial fulfillment for the award of Higher National Diploma (HND) in Science Laboratory Technology

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DEDICATION

This research project is dedicated to God Almighty for His endless grace, wisdom, and strength throughout this journey.

I also dedicate this work to my beloved parents, whose unwavering support, encouragement, and sacrifices have been the foundation of my academic pursuit.

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My thanks goes to almighty God for all his protection, grace,and blessings upon me for the successful completion of this project work and my course of study.

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My sincere and immeasurable gratitude is attributed to my dearest and irreplaceable parent MR and MRS KAYODE for their financial, spiritual, moral and credible support over my life, may you live long to eat the fruits of your labor (Amen).

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And finally to all lecturers in science laboratory technology, may God continue to bestow his mercy onto you all (Amen).

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ABSTRACT

In this study silver nanoparticles were successfully synthesized using Gossypium arboreum aqueous leave extract. The effect of the whole extract and some of its phytochemical on the partical distribution was determined while other characterization such as UV-VIS spectrophotometer, fourier transformed infrared

(FTIR) spectroscopy, scanning electron microscopy (SEM) were carried out. The antimicrobial activity against selected microorganism was also studied using the agar diffusion method. The leaf extract showed the presence of phenols, flavonoids, saponins, steroids, terpenoids, alkaloids, and tannins. The UV-VIS spectra displayed the characteristics Plasmon resonance at 350nm, the FTIR plot highlighted important functional groups including peaks between 3776.22cm^{-1}, 2262.00cm^{-1}and 903.38cm^{-1} respectively confirming the formation of silver oxide. After characterization,the silver nanoparticles were tested at various concentration to check their antimicrobial activity against clinical isolates of different bacteria and fungi. Gossypium arboreum/AgNO3 played significant antimicrobial activity against penicilum spp, Aspergilus niger, Trichoderma spp, fusarium spp, Rhizopus spp, Klebsiella spp, Pseudomonas spp, while no activity was recorded for Escherichia coli, Staphlococus, aureus and salmonella typhi. Maximum zone of inhibition of bacteria was observed against Pseudomonas spp(28mm at 1000ppm)and minimum zone of inhibition of bacteria was observed against Klebsiella spp(21mm at 1000ppm). While maximum zone of inhibition of fungi was observed against Rhizopus spp(16mm at 0.5ml) and minimum zone of inhibition of fungi was observed against Fusarium spp(10mm at 0.5ml). The study indicated that Gossypium arboereum was suitable for the synthesis of silver with properties that suggested the potential used of this plant extract in developing natural antimicrobial agent.

CHAPTER ONE

1.0 INTRODUCTION

Nano word originated from latin word, which means dwarf ideal size range offered by nanotechnology refers to one thousand million of a particular unit thus nanometer is one thousand millionth of a meter (i.e 1nm=10^{-9}m). the branch nanotechnology is the science that particularly deals with the process that occur at molecular level and of nano length scale size. Nanotechnology is now become an allied science which is most commonly used in other field of science like electronic, physics and engineering since many decades (*laxman et al,* 2021)

Silver nanoparticules have been reported to exhibit number of pharmacological activities, including antibacterial activity, antifungal activity, anticancer activity, antiviral activity (*rajkumar et al., 2011*)

Since most of these chemically synthesized nanoparticules are utilized for human benefits, they pose severe hazard to human health and to the environment. Recently, green synthesis of nanoparticles using extracts of plants and microbes have gained importance because it could solve the problem of toxicity imposed by chemical methods. Green synthesis of silver nanoparticles using plants which includes *calotropis procera*, *Bryophylum pinnatum*, *Jatropha curcas*, the studies have shown that silver nanoparticlescan be synthesized in environmental friendly and green method using plant extracts. The synthesis of nanoparticles has been carried out using different approaches, including physical and chemical methods. In physical methods, nanoparticles are prepared by evaporation condensation using a tube furnace at atmospheric pressure. Chemical methods uses water or organic solvent to prepare the silver nanoparticles, each traditional plant has specific medicinal properties; by employing these plants the medicinal property of the synthesized silver nanoparticles may be enhanced.

In contrast with these methods, biological method e.g plant extracts, bacteria and fungi are known to be safe as they utilize very fewer toxic reactants or additives. This method is also considered to be rapid, simple, user-friendly and inexpensive and includes the capability of synthesis in large quantities (*rajeshkumar et al., 2017*). The synthesis of nanoparticles using biological source has gained interest in recent days. The application of plant extracts is highly recommended for the production of silver nanoparticles (*pantidos et*

al., 2000) extracts from plants material are high in secondary metabolites including enzymes, polysaccharides, alkanoids, tannis, phenols, terpenoids and vitamins which allow them to display excellent antimicrobial properties (*cowan. M.m 1999*).

It is assumed that organic components from the leaf extract (flavonoids and terpenoids) helps to stabilize the silver nanoparticles synthesized using *Gossypium arboreum* leaves could be biocompatible and can be used for biological applications.

Gossypium arboreum belongs to the malvaceae family. The *Gossypium arboreum* is most important species of the family. It has gained great importance due to its pharmacological properties in including antimicrobial activity against most of the microorganisms. The G.*arboreum* has phytochemicals such has alkaloids and glycosides, flavonoids, phenols, saponins and tannins. The phenolics, alkaloids and glycosides have antimicrobial activity. Alkaloids, saponins and tannins show strong antimicrobial activity with various antibiotics against various bacteria. So development of Gossypium *arboreum* silver nanoparticles may show the better anti-microbial activity than the extract (*nagarajan k, et al., 2014*).

The aim of this study was to synthesize and characterize plant-mediated silver nanoparticles using *Gossypium arboreum* extract and evaluate their antifungal and antibacterial activity. This research aims to explore the potential application of green-synthesized AgNps in biomedical fields.

OBJECTIVES OF THE STUDY

▪ to synthesize silver nanoparticles using *Gossypium arboreum* leaf extract

▪ to characterize the synthesized nanoparticles using techniques such as UV.VIS spectroscopy, SEM, FTIR.

▪ To evaluate the antimicrobial activity of the synthesized AGNPS against some bacteria.

▪ To evaluate the antimicrobial activity of the synthesized AGNPS against some fungal strains.

SIGNIFICANCE OF THE STUDY

Gossypium arboreum is one of the most commonly grown varieties. The distribution of compounds within the *Gossypium* genus varies across

different part of the cotton plant and the variance corresponds to their distinct properties and functions within the plant (*egbuta m.a et al., 2017*). *Gossypium arboreum,* specifically shows potential in treating malaria as well as managing associated symptoms such as pain and inflammation.

Also used in preventing and treating conditions such as oral cardidiasis (*balliana R.C et al,. 2014*). Respiratory ailments reproductive health issues and geniotourinary conditions.

Traditional use for its leaves and roots involve decocting them to address digestive disorders like diarrhea and dysentery. Additionally the decoction and infusion of cotton leaves are employed for treating injuries and diarrhea (*umar et al,. 2023*).

PROBLEMS OF THE STUDY

1. Variability in nanoparticles synthesis methods and outcome
2. Limited studies on *Gossypium arboreum* mediated green synthesis of silver nanoparticles
3. Need for more research on antimicrobial application of green synthesized AGNPS

1.1 LITERATURE REVIEW

In science and engineering, sustainable nanotechnology is successful in giving solutions for the challenges in various sectors such as medicine, catalysis, industrial and agricultural activities, nanotechnology and nanoparticles has advanced in such a way that they are even capable of producing entities possessing bio like functions. Microscopic particles with diameter less than 100nm are called nanoparticles (*ariga,K, 2017*)

Due to their incredibly small size (measured in nanometers) and elevated surface to volume ratio which result in both physical and chemical changes in their properties when compared to most materials with the same make up, nanoparticles are of great interest. Inorganic nanoparticles includes semiconductor nanoparticles (*zns, cds, zno*) metallic nanoparticles (*Ag,Av,Al,Gu*) and magnetic nanoparticles (*CO,NI,FE*) while organic nanoparticles contains carbon nanoparticles (*fullerences, yakut S.M et al,.*

2021). Due to their ecological compatibility which is attributed to the past few decades, green metallic nanoparticles centered goods have become increasely accepted and popular in research given the wealth of resources found in the biosphere that fit into this category a wide range of goods would always be available to fulfil the need for green (*ponsati k et al,. 2022*). Plants have found use in the manufacture of metal NPS, specifically in the field of nanoparticles synthesis involving living organisms. (*jayeoye T.J et al,. 2021*). Compared to other biological methods that are safe for the environment, using plant to synthesize nanoparticles could be advantages because it reduces the complex procedure of preserving cell cultures. It would be more beneficial for biosynthetic processes to make nanoparticles extracellularly from plants or their extract in a regulated way that considers their magnitude, dispersity and form (*priyadarshini j.f et al,. 2018*).

One form of nanomaterial with several uses in food packaging industrial operations, medicine and water treatment is silver nanoparticles (*mohammed f et al,. 2019*).

Their remarkable electrical, thermal optical and biological characteristic which also bear similarities to those of noble metals like copper and gold set them apart from other metalions (*syafiuddin A et al,. 2017*).

Metal nanoparticles are astronomically exploited on account of their distinctive properties like reactivity, physical properties and probable application in enormous research areas like anti-microbial, diagnostic antioxidant and specific drug delivery (*singh A et al,. 2019*). The silver nanoparticles antibacterial activity is more potent the smaller the silver nuclei, thus size management and size distribution are essential characteristics of higher performing end goals. Changing the stabilizers and reducing agent during preparation is a common technique to regulate the size and distribution of silver nanoparticles (*shayo G.M et al., 2024*).

Research has demonstrated that phenolids, flavonoids, and glycosides, among other various bio-active metabolites are abundant in natural goods and can easily reduce metallic ions when combined in the similar reaction vessel. This category comprises polymers such as 3-*amonibenzene boronic* acid groups, polyethylene glycol, *polyvinyl pyrrolidone*, *polyvinylalcohol* and *pluronic groups (Aung Y.Y et al,. 2021).*

1.2 GREEN SYNTHESIS OF SILVER NANOPARTICLES

The choice of a safe stabilizing substance an effective reducing agent and an environment friendly solvent are the 3 crucial requirements for the preparation of nanoparticles many synthetic techniques including physical, chemical and biosynthetic processes, have been used to synthesize nanoparticles. The chemical methods that are usually used are very costly and include the usage of risky and deadly materials that represent several environmental risk (*Razavi M. et al,. 2015*). The biosynthetic way is a green, unharmed and ecologically acceptable way to create nanoparticles for use in biomedical applications using microbes and plants. Among other things, algae, fungi, bacteria and plants can be employed to carryout this synthesis. Many nanoparticles have been synthesized from plant parts such as leaves, fruits, roots, stems and seeds because these plant parts include photochemicals that function as stabilizing and reducing agents in the extract. Numerous biological and physiochemical processes for the creation of nanoparticles can be divided into two distinct classes: top-down and bottom-up (*rajkumar T er al,. 2019*). Figure 1a and b illustrates the development of nanomaterials. The top-down approach is predicted on the idea of using chemical or mechanical interventions to reduce large size materials nano-size unlike the physical top-down strategy, which is based on chemical reactions, the bottom-up approach creates atoms or molecules through a variety of chemical reactions. Some other conventional methods (physical and chemical) along with green synthesis method are shown in fig.1b (*karatas E. et al., 2023*).

Fig 1a Fig 1b

1.2 FACTORS AFFECTING SYNTHESIS

Certain physiochemical characteristics, including temperature, time, PH, optical, substrate concentration and enzyme sources influences the creation of nanoparticles. The information in the table below provides the explanation of different factors that affect the synthesis of silver nanoparticles.

Table 1 Factors that affect the green synthesis methods

Factors	Description	Impact on NPs synthesis
pH	How acidic or alkaline the reaction media is	pH has an impact on the nucleation and growing processes of NPs by changing the charge on their surfaces. Smaller particles are often produced by higher pH, but aggregation can occur at lower pH
Temperature	The temperature during the process of synthesis	Greater heat can cause aggregation; lower temperatures promote nucleation and reduction rates, resulting in lesser and more homogeneous NPs
Concentration	The quantity of reducing agent and metal precursor	Reducing agent amount impacts stability and reduction rate higher Precursor amounts enhance nucleation sites, producing reduced atoms but can also promote accumulation
Time	Time periods of reaction process	Affects the development and maturation of NPs Larger, more definite shapes might result from longer durations, but too much time can also lead to aggregation and polydispersity

Light intensity	Crucial factor that significantly impacts the synthesis of SNPs	Since UV light gives off energy that accelerates the reduction of silver ions, its function is very note worth

2.2.1 Effect of temperature: the primary physical factor influencing nanoparticles production is temperature, according to report, AG$(NO_3)_2$ and starch solution can be autoclaved at 121^0c and 15 psi to create silver nanoparticles (*galindo H.M et al,. 2011*). Ag and ion nanoparticles were synthesized at standard room temperature utilizing plants extracts, like aqueous extract of sorghum bran according to (*galindo et al. 2011).* The

temperature also affect the additional stability of the nanoparticles. The silver nanoparticles produced are kept between 18 and 25^oc for two to three months (*Tamulalliene A et al, 2009*).

2.2.2 Effect of concentration of the substrate and the reducing agents: silver nanoparticles can be influenced b amount of plant extract and salt concentrations (*hasnain et al, 2019*). Ag nitrate ($AGNO_3$) is often employed as a precursor for the production of NPS by using Ag ions. The size and rate of the nanoparticles is greatly influenced by the quantity of salt present in the solution. Larger nanoparticles sizes are frequently the outcome of higher salt concentrations since there are additional Ag oins available for carrying out the reduction conversely, low concentration of the salt can produce smaller nanoparticles (*khan S. et al, 2024*).

2.2.3 Effect of PH: another physical element which influence the morphology (size and shape) is PH particle size is influenced by structural difference between disaccharides and monosaccarides. At PH 11.5, disaccharides in this reaction often yield smaller particles than monosaccharides. Furthermore, compared to particles formed at PH 12.5, those obtained at PH 11.5 were smaller. One way to reduce polydispersity is to lower the reaction mediums PH (*Sharma et al,. 2009*).

1.4 PREVIOUS REPORTS ON THE SYNTHESIS OF AgNP

Many research work have been done on the synthesis of AgnPS when bulk silver is reduced to nanosilver, it exhibit remarkable changes in its different properties, making it more valuable from the application point of view. Since then, many researchers have synthesized AgNPs using different methods some researchers utilized AgNPS to incorporate them into cotton fibres to investigate antibacterial activity against some bacteria. Some of the works are discussed below.

M.C *lea* synthesized the first nanosilver or silver nanoparticles in 1889. He used citrate to stabilize AgNPs and the average size was between 7 to 9nm (*nowalk et al,. 2011*).

The pulsed laser ablation method is one of the most popular techniques for synthesizing AgNPs. *Zamiri et al.* prepared AgNPS in natural polymers assisted by the pulsed laser ablation method. An Ag plate (99.99% purity) was irradiated by a Q switched nel: YAG pulsed laser of wavelength 532nm and energy 360mj/pulse. Silver atoms were ejected from the metal surface

by local high temperature and plasma plumes of high pressure. Then the ejected silver ions formed AgNPs through coeling and condensation. Different polymers such as gelatin, starch and chitosan were used as stabilizers. It is a rapid and simple one-step process of preparation of AgNPs and provides a significant result. They found minimum sized nanoparticles using as gelatin as a stabilizer. *Wang et al,. 2005,* reported the chemical synthesis of AgNPs using $AgNo_3$, glucose, polyuinyl pyrrolidone (PVP) and sodium hydroxide. They synthesized AgNo3 by reducing AgNo3 in pvp, glucose and NaoH aqeous solution, the synthesized AgnPs were well dispersed with sizes ranging from 20-80nm, suggesting a wide range size distribution. It is crucial to maintain a suitable amount of dispersant, which controls the formation and agglomeration of AgNps. If there is a lack of dispersant which controls the formation and agglomeration of AgNPs. If there is a lack of dispersant, the protection layer cannot be formed, leading to aggregation. However, this is a multi-step and costly process and requires many chemical reagents. Some of them are toxic chemicals and hazardous to our environments. Sometimes bi-product come out from different reactions, which are of no use except for polluting the surroundings, so this problem led researchers to prefer the biological route to develop an eco friendly cost effective and one step process.

In the biological route, various species of fungus are widely exploited for the biogenic synthesis of AgNPs.

Dameron et al,. first utilized candida albicans fungus to synthesis cdse Nps in 1989.

Guilger-casagrande et al 2019. Synthesized AgNPs employing *trichoderma harzianum* fungus. At room temperature, they cultivated T.harzianum using wettable powder in potato dextrose agar medium. This fungus culture was done in the absence of light and the temperature was maintained nearly constant. Their report confirmed that the mean diameter of biosynthesized AgNPS was 57.02± 1.75nm and the zeta potential value was -18.70±3.01mv. biological synthesis using fungi is very length process. It take 12 to 15 days to cultivate fungi and requires extra carbon and nitrogen for growth. Then additional three days are needed to prepare fungus as a reducing agent. The reduction rate is slow and the particles size obtained was larger than plant mediated synthesis.

Kajani et al. (2014),synthesized anisotrophic AgNPs using *Taxuz baccata* extract and investigated their potent anticancer activity. They performed several characteristics to study the physical, chemical thermal and cytoxic properties of synthesized anisotropic AgNPS from particle size distribution, the average size of anisotropic AgNPs was found to be 91nm. The zeta potential value was measured as -77mv for optimal conditions.

Florence o. et al,. (2013), made a research on the production characterization and application of silver nanoparticles (AgNPs) which can be utilized in biomedical research and environmental cleaning applications. They used an environmentally friendly extracellular biosynthetic technique for the production of the AgNPs. The reducing agents used to produce the nanoparticles were from aqueous extracts made from the leaves of various plant. Synthesis of colloidal AgNPs was monitored by UV. Visible spectroscopy. The UV-visible spectrum showed a peak between 417 and 425nm corresponding to the Plasmon absorbance of the AgNPs. The characterization of the AgNPs such as their size and shape was performed by atom force microscopic (AFM) and transmission electron microscopy (TEM) techniques which indicated a size range of 3 to15nm. The anti bacterial activity of AgNPs was investigated at concentration between 2 and 15 ppm for gram-negative and gram positive bacteria. The results indicated that AgNPs at a concentration of 2 and 4ppm, inhibited bacterial growth.

Sumitha et al (2021), synthesized AgNPs using *spondias mombin* extract. The synthesized AgNPs were characterized and further tested for their antimicrobial, reactive oxygen species and cytotoxicity properties. The characterization result showed the synthesized AgNPs to be between 8 to 50nm with -11.52 of zeta potential value. The existence of the silver element in the AgNPs was confirmed with the peaks obtained in the EDX spectrometry significant antimicrobial activity was observed against selected bio-film forming pathogenic bacteria. The cytotoxicity study with A. *salina* revealed the 1050 of synthesized AgNPs was at 0.81mglml. this proves that the synthesized AgNPS could be an effective drug against multidrug resistant bacteria.

Arikiyaraj et al,. reported the synthesis of AgNPS using *chrysanthemum indicum (c. indicum)*floral extract and studies its antibacterial and cytotoxic effects. They also characterized size the optical and morphological properties of synthesized AgNPs. Their TEM micrograph revealed that the average size AgNPs ranged from 37nm to 71nm. EDS analysis confirmed that AgNPS were crystalline and exhibited face centered cubic structured XRD analysis showed characteristic peaks at 38° ,44°,64°.$77^{\circ}$and 81° corresponding to 111,2000,220,311 and 222 planes. The crystallite size calculated from the XRD pattern using scherrer's equation was ~53nm. The size range distribution suggested that synthesized AgNPs had a wide size distribution. They tested antibacterial efficiency against both gram positive and negative gram bacteria. The result showed a zone of inhibition of 8.33± 057nm against staphylococcus qureus (*s. qureus*) and 13±0.90nm against escherichacoli (*E.coli*) bacteria. This better efficacy against gram negative bacteria was due to the thickness peptidoglycan layer.

Many researcher synthesized nanoparticles using plant grown under in vitro conditions.

Pirtarighat et al. synthesized nanoparticles using the green route where they used plant extract of *salvia spinosa* in vitro. First, the seeds of *salvia spinosa* were sterilized with 70% ethanol followed by 2.5% sodium hypochlorite petri dishes were explanted for germination synthesized AgNPs were characterized with FESEM, FMR and XRD analysis. The synthesized AgNPs exhibited good efficacy against gram positive and gram negative bacteria. But harvesting salvia spinosa in vitro is a sophisticated and lengthy process. It requires about two months, according to their report. The

XRD pattern of their synthesized AgNPs confirmed crystalline structure, but there were few undefined peaks with high intensity. It may due to impurities which can be a barrier to the proper use of AgNPs.

The surface morphology of synthesized AgNPs shows agglomeration. The average particle size was calculated from the FESEM image and DLS analysis. The average particle size was 19 to 125nm in diameter according to FESEMA and from DLS analysis, it was 5.13nm. these measurements were inconsistent with each other. Moreover, synthesized AgNPs had a wide range of size distribution.

Roy et al. reported the synthesis of AgNPs using *Azadirachta indica* and investigated their antimicrobial activity. Their study suggested that the absorbance of the synthesized AgNPs increased with the increase of leaf extract ratio with respect to $AgNO_3$. The average particle size obtained from DLS analysis was 65-67nm in diameter and the polydispersity index varied from 0.280 to 0.299. DLS analysis revealed a wide particle size distribution. They tested their synthesized AgNPs against both gram positive and gram negative bacteria. They applied 20,50 and 100nl of synthesis AgNPs solution into the diffusion well. The 20nl varied from 8.66mm to 22mm depending on the amount of AgNPs solution used in a diffusion well and the condition under which the AgNPs were synthesized. They got a better antibacterial response against gram negative bacteria and results varied from 11.83 to 16.67mm.

Rao et al. reported the synthesis of AgNPs using *Eriobotrya japonica* extract and investigated antibacterial activity. They perform optical, morphological, structural and elemental characterizations. The synthesized AgNPs were spherical with a narrow size distribution ranging from 46nm to 70nm. They also studied the effect of reaction temperature on particles size.

The average particle size was 76.1nm synthesized at 25°c, where as AgNPS synthesized at 80°c had an average size of 54.47nm in diameter. The zeta potential value of -20.4mv indicated moderate dispersion stability. They tested the antibacterial efficiency of biosynthesized AgNPs against E.coli and S. qureus bacteria strains. Their result showed that zone of inhibition
was about 4.5nm for 100mg/l concentration of AgNPs against staphylococcus aureus bacterium, this antibacterial efficacy is lower compared with some other reports.

In another study, AgNPs were synthesized by using tea leaf extract. Bactericidal activity of the synthesized nanoparticles was tested against *S. aureus and E. coli* showed that inhibition action is more effective in case of *s.aureus (*89% inhibition rate*)*compare to *E .coli (*75% inhibition rate*)*. In addition, treatment of the nanoparticles against the bacteria leads to impairment of bacterial cell adhesion (*Goswami S.R et al., 2015*).

Mukia maderaspatana leave extract was utilized for the biosynthesis of silver nanoparticles with the size range of 58-458nm. The synthesized nanoparticles was conjugated to the antibiotic ceftriaxone to investigate the antimimcrobial activity towards the human pathogens such as *B. subtilis, k. pneumonia, s. typhi, s. aureus and* compared with the pathogen inhibition efficiency of the free nanoparticles and the antibiotic. The result obtained revealed that the silver nanoparticles conjugated with ceftriaxone showed highest inhibition activity compared to the others (*M.Harshiny et al., 2015*).

CHAPTER TWO

2.1 MATERIAL AND METHOD

In this chapter, the essential steps for synthesizing AgNPs using plant mediated green synthesis are presented initially. Later on, the characterization techniques to investigate the optical, structural, morphological, thermal and antibacterial properties of AgNPs have been discussed

2.2 COLLECTION AND IDENTIFICATION OF PLANT EXTRACT AND BACTERIAL ISOLATES

Gossypium arboreum leaves were collected in Ganmon area and clinical isolates of *staphylococcus aureus, salmonella spp, Escherichia coli, klebsiella spp, pseudomonas spp* were all collected.

2.3 PREPARATION OF AQUEOUS SILVER NITATE ($AgNo_3$)

A 5mm AgNo3 (silver trioxonitra (v)) solution was prepared by weighing 0.085g of AgNO3 using an analytical balance and dissolving it in 100ml of distilled water of a 100ml volumetric flask to reach the 100ml mark (Debabrat et al,. 2012).

PREPARATION OF AQUEOUS AND ETHANOLIC *GOSSYPIUM ARBOREUM* EXTRACT AND AgNPs SYNTHESIS.

60g of Gossypium *arboreum* leaves was weighed and rinsed with running tap water several time to remove dirt, dust and other contaminated organic chemicals from the surface of the leaves. Then the leaves were finally washed with distilled water and finely chopped into fine pieces. The chopped leaves were mixed with 100ml distilled water in a *Erlenmeter* flask and the mixture was boiled for 1 hour at 60°c. the mixture was cooled and then filtered with whatman paper no 1. The filtrate was collected and stored at 4° c for further analysis.

Ethanolic extract was prepared by taking 60g pulverized leave of Gossypium *arboreum* in 100ml of ethanol in 250ml conical flask and kept for 24 hours in rotary flask shaker and extract was filtered by *whatman filter* paper no.1 and stored for further use.

The 5ml of leaf extract was added to 45ml of 5mm $AgNO_3$ solution and 5ml of ethanolic leaf extract was added to 5mm of $AgNo_3$ in Erlenmeyer flask

for bioreduction process at 30°c in dark and observed for change in colour (*debabrat et al,. 2012*).

PHYTOCHEMICAL ANALYSIS OF ACTIVE COMPOUNDS IN THE LEAVE EXTRACT

Phytochemical screening was performed using standard procedures by using different specific reagents, the presence of main groups of natural products was detected in the ethanolic extracts of *Gossypium arboreum*

FLAVONOIDS

A portion of the extract was dissolved in 2ml of 50% methanol. Metallic magnesium chips and a few drops of concentrated hydrochloric acid were added. The appearance of a red colour indicates the presence of flavonoids (*namadina et al,. 2020*).

ALKALOIDS

To a few milliliters of the plant sample extract, two drops of mayer's reagent are added using the sides of the test tube. The appearance of a white creamy precipitate indicates the presence of alkaloids (*Banu and Catherine, 2015*).

SAPONINS

5ml of the extract sample was diluted with 15ml of distilled water. The resultant mixture was shaken strongly, the appearance of foam indicates the presence of saponins (*Oscar et al., 2020*).

TERPENOIDS

A small sample of the extract was treated with 1ml of acetic anhydride, 1ml of trichloromethane and 1 ml of sulfuric acid. The production of a violet color indicates the presence of terprnoids (*Oscar et al,. 2020*).

GLYCOSIDES

Glycosides were detected by adding a few drops of glacial acetic acid, ferric chloride, and concentrated sulfuric acid to each sample through the side wall of the junction of the two layers and the upper layer appeared bluish-green (*rajitha et al,. 2022*).

TANNINS

50mg of the extract is dissolved in 5ml of distilled water to this a few drops of neutral 5% ferric chloride solution are added. A dark green color indicates the presence of phenolic compounds, for the detection of tannins, 2ml of the extract is placed in a test tube and gently heated for 2 minutes. An orange color observed after adding 3 drops of ferric chloride indicate the presence of tannins (*Rashed et al,. 2019*).

STEROIDS

Salkowski test: extract were treated with chloroform and filtered. The filtrates were treated with a few drops of concentrated sulfuric acid, shaken and allowed to stand. The appearance of a golden yellow color indicates the presence of triterpenes (*khanal et al,. 2015*).

2.4 CHARACTERIZATION OF SILVER NANOPARTICLES (AGNPS)

2.4.1. UV SPECTROPHOTOMETER ANALYSIS OF SILVER NANOPARTICLES

The bioreduction of Ag^{+}ions in solution was monitored by measuring the UV-VIS spectrum of the reaction medium. The UV-VIS spectral analysis of the sample was done by using U-3200 *Hitachi* spectrometer at room temperature operated at a resolution of 1nm between 200 and 600nm ranges. Distilled water was used as blank for silver nanoparticles (*debabrat et al.2012*).

2.4.2 SEM ANALYSIS OF SILVER NANOPARTICLES

Scanning electron microscopy (SEM) study was carried out by focusing electron beam over a surface to create an image.

The electron in the beam interacts with the sample, producing various signals that can be used to obtain information about the surface topography and composition. The type of signals produced by a SEM include secondary electron (SE) back scattered electrons (BSE), characteristic Xrays, light (*cathodoluminescence*) (CC), specimen current and transmitted elections secondary electron detectors are standard equipment in all SEMs, but it is rare that a single results from interaction of the electron beam with atoms at or near the surface of the sample. The SEM can produce very high resolution images of a sample surface, revealing details less than 1nm in size.

Back-scattered electrons (BSE) are beam electron that are reflected from the sample by elastic scattering characteristics X-rays, are emitted when the electron beam removes an inner shell electron from the sample, causing a higher-energy electron to fill the shell and release energy, these characteristics X-rays are used to identify the composition and measure the abundance of elements in the sample.

SAMPLE PREPARATION

All sample must be of an appropriate size to fit in the specimen chamber and are generally mounted rigidly on a specimen holder called a specimen stub. Several models of SEM can examine any part of a 6-inch (15cm) semi conducted water, and some can tilt an object of that size to 45^{o}.

Samples are coated with platinum coating of electrically conducting material, deposited on the sample either by low vacuum sputter coating or by high-vacuum evaporation. SEM instruments place the specimen in a relative high-pressure chamber where the working distance is short and the election optical column is differentially pumped to keep vacuum adequately low at the electron gun. The high pressure region around the sample in the SEM neutralizes charge and provides an amplification of the secondary electron signal low-voltage SEM is typically conducted in an FEG-SEM because the field emission guns (FEG) is capable of producing high primary electron brightness and small spot size even at low accelerating potentials. Embedding in a resin with further polishing to a mirror-like finish can be used for both biological and materials specimens when imaging in backscattered electrons or when doing quantitative X-rays microanalysis.

2.4.3 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) ANALYSIS

Fourier transform infrared spectroscopy (FTIR) analysis was performed in all samples isolated to have a prompt result regarding the bio mineral. A few crystals were mixed with KBr (*merck for* *spectoscopy*) and pulverized in a agate mortar to form a homogenous powder from which under a pressure of 7 tons, the appropriate pellet was prepared. All spectra were recorded from 4000 to 400cm^{-1} using the *pelkin elmer* 3000 Mx spectrometer scan were 32 per spectrum with a resolution of 4cm^{-1}. The IR spectra were analyzed using the spectroscopic software Win-IR pro version 3.0 with a peak sensitivity of 2$cm^{-1.}$

2.4.4 ANTIMICROBIAL ACTIVITY

Antimicrobial activity of Gossypium *arboreum* leaves extract (*aqueus & ethanolic*) and its synthesized silver nanoparticles were evaluated by agar well diffusion method, using two test gram positive organism such as *staphylococcus aureus* and *Escherichia coli*, two gram negative bacteria *klebsiella pneumoniac* and *pseudomonas aeruginosa* and five fungus.

Aspergillus niger, rhizopus spp, trichoderma spp, penicillum spp, fusarium. Inoculums of test organisms turbidity was adjusted and culture used were 24hours fresh culture inoculated on a surface of agar plates with sterile cotton swab, agar plates were incubated at 37^{o}c for 24hours for bacteria and at 28^{o}c for 24hours for fungal strains. After 24h wells of 6mm diameter was made with sterile pore borer and wells were loaded with 10g/ml of silver nanoparticles, 5mm silver nitrate and plant extract after 24hours of incubation efficacy of silver nanoparticles, silver nitrate and *Gossypium arboreum* leaves extract was determined in terms of zone of inhibition.

The antibacterial activity was evaluated by measuring the diameter of the resulting zone of inhibition against the tested microorganisms in millimeters.

CHAPTER THREE

3.1 RESULTS

GREEN SYNTHESIS OF SILVER NANOPARTICLES

The study showed a change in the color of the solution of *Gossypium arboreum* leave extract will form a brown solution after mixing $AgNO_3$. The

discolouration is an indication that Ag^{+} has been reduced to AgNPs in the solution.

PHYTOCHEMICAL ANALYSIS

The results from the quatitative photochemical analysis of *Gossypium arboreum* leaf extact showed the presence of alkanoids and flavonoids which are highly present saponins and steroids are present in trace form. This revealed the presence of various bioactive secondary metabolites, confirmed through chemical color reaction tests as shown in table 1&2.

Table 1.

Result of qualitative phytochemical screening of *Gossypium arboreum* leaf

S/N	PHYTOCONSTITUENT	G. *arboreum* extract
1	Flavonoids	+ + +
2	Alkaloids	+ + +
3	Saponin	+ +
4	Terpenoids	+
5	Glycosides	+

6	Tannins	+ +
7	Steroids	+

Key

+ present in trace form

+ + moderately present

+ + + highly present

Table 2:

Result of quantitative phytochemical screening of *Gossypium arboreum* leaf

Phytoconstituents	G.arboreum leave extract
Flavonoids (mg/100g)	20.053±2.859
Alkaloids (mg/100g)	22.885±0.922
Saponins (mg/100g)	14.473±0.783
Terpenoids (mg/100g)	1.088±0.092
Glycosides (mg/100g)	5.303±0.166
Tannins (mg/100g)	7.208±0.149
Steroids (mg/100g)	1.878±0.073

FTIR ANALYSIS

The FTIR spectrum as shown in fig 1 of *Gossypium arboreum* silver nanoparticles showed a strong IR bands characteristics of hydroxyl (3471.00cm ^{1}), alkanes (4047.00cm ^{1},3776.22cm ^{1}, 2929.76cm ^{1}), c=c of benzene (2363.00cm^{-1}, 2262.00cm ^{1}, 2149.00cm ^{1}) aromatic amines (1628.72cm^{-1}, 1383.00cm^{-1}, 1316.38cm^{-1}, 1258.45cm^{-1}, 1079.70cm^{-1}, 1026.51cm^{-1}, 903.38cm^{-1}) and aliphatic amines (608.25cm^{-1}, 477.90cm^{-1}, 369.50cm^{-1}) functional group

53.1 52
50
48
46
44
42
40
38
36
34
32
3776.22

2262.00
2363.00

2149.00

1628.72

1258.45
1316.38
1383.00
1079.70

903.38

608.25
1026.51

477.90
369.50

%T 4047.00 30
28
26
24
22
20
18
16
14
12.0

3471.00
2929.76

3419.19

4400.0 4000 3600 3200 2800 2400 2000 1800 1600 1400 1200 1000 800 600 350.0
cm-1

Fig 1: FTIR spectrum analysis.

SEM ANALYSIS

The presence of silver nanoparticles capped phytoconstituent was confirmed. Our experiment reacts showed the synthesized silver nanoparticles were found to be spherical in shape and they are quite well distributed without any agglomeration as shown in fig 2

Fig 2: SEM analysis

UV- SPECTROPHOMETER ANALYSIS

The synthesized aqueous silver nanoparticles showed strong absorption 3. Shows the UV-visible absorption spetra of the silvernanoparticles formed band at 350nm which was due to the surface Plasmon resonance (SPR). Fig

26

at different time intervals from aqueous solution of silver nitrate with *Gossypium arboreum* extract

2.443

2.440

2.406

2.404

2.395

2.393

2.378

2.376

2.372

2.370

2.364

2.362

2.358

2.262

2.260

0.000

200 250 300 350 400 450 500 550 600 650

ANTIMICROBIAL ACTIVITY

In the present study, we observed the silver nanoparticles synthesized using Gossypium arboreum extracts showed a pronounced zone of inhibition. This is proved by the values of diameter of zone of inhibition obtained in table 1 and 2. It is apparent that the silver nanoparticles showed inhibition zone against almost all the test organism, the synthesized AgNPs were found to have higher inhibition action

Table 1: antimicrobial activity of silver nanoparticles of Gossypium arboreum counter to gram positive and gram negative bacteria

Zone of inhibition (mm) of silvernanoparticles

Bacteria	250ppm	500ppm	1000ppm
E.coli	-	-	-
Klebsiella spp	--	25mm	21mm
Pseudomonas spp	-	-	28mm
Staphylococcus aureus	-	-	-
Salmonella typhi.	-	-	-

Table 2:antimicrobial activity of silver nanoparticles of Gossypium arboreum counter to fungi.

Zone of inhibition (mm) of silver nanoparticles

Fungi	0.2ml	0.5ml	1ml
Penicillum spp	-	13mm	15mm

Aspergillus Niger	-	12mm	14mm
Trichoderma spp	-	11mm	15mm
Fusarium spp	-	10mm	14mm
Rhizopus spp	-	16mm	14mm

CHAPTER FOUR

4.0 DISCUSSION

The silver nanoparticles (AgNPs) synthesis was carried out using Gossypium arboreum leave extract. The reaction carried out in a dark condition to avoid the photo activation reaction of silver nitrate (*Sharma et al,.* 2019). An indication of AgNPS formation is the occurrence of discoloration in the solution. After some incubation time, it was observed that the AgNPs solution has changed to brownish color. The discoloration is an indication that Ag^+ has been reduced to AgNPs in the solution (*Sharma et al,. 2019*). The study showed a change in the color of the solution after 48 hours of incubation. A light green solution of *Gossypium arboreum* leave extract will form a brown solution after mixing $AgNO_3$.

The preliminary photochemical screening conducted on the ethanol extracts of *gossypuim arboreum* leaves revealed the presence of various bioactive secondary metabolites, confirmed through chemical color reaction tests as shown in table 1&2. The result from the qualitative and quantitative photochemical analysis of *Gossypium arboreum* leave extracts showed the presence of alkaloids and flavonoids which are highly present saponins and tannins are moderately present while terpenoids, glycosides and steroids are present in trace form. These findings are consistent with AI-snafi (2018) and *patilet et al,. 2014.*who found that *Gossypium arboreum* contained alkaloid,

phenolic compounds, terpenoids, tennins, saponins, flavonoids, cardiac glycosides and proteins. The phytochemical analysis conducted in this study indicated that G. *arboreum* leaves also contained bioactive compounds, which are the primary basis for the medical properties of the plant. *Ayeni et al.* reported that the presence of and medicinal properties of secondary plant metabolites such as alkaloids, saponins, glycosides, flavonoids and tannins in the leaves of *Gossypium arboreum* as reason why they are used as medicinal plants (*ayeni et al., 2015*).

FTIR measurement was carried out to identify the possible biomolecules in *Gossypium arboreum* responsible for capping leading to efficient stabilization of the silver nanoparticles. FTIR spectroscopy is used to identify the functional groups that play an important role in reducing and stabilizing the silver nanoparticles prepared using *Gossypium arboreum* leaf extract. The FTIR spectrum (fig 1) of silver nanoparticles showed strong 1R bands characteristics of hydroxyl (3471.00$cm^{-1}$and 3419.19cm^{-1}) alkanes (4047.00cm^{-1}, 3776.22$cm^{-1,}$ 2929.76cm^{-1}), c=c of benzene (2363.00cm^{-1}, 2262.00cm^{-1}, 2149.00cm^{-1}) aromatic amines (1628.72cm^{-1}) and Aliphatic amines (608.25 cm^{-1}, 477.90 cm^{-1}, 369.50 cm^{-1}) functional groups. Change in the position and intensity distribution of 1R bands in the spectra reveals phytoconstituents for bio reduction and stabilization of silver nanoparticles (*nagarajan et al,. 2014*).

Primary electron can produce several secondary electrons, thus secondary electrons are abundant and the most used imaging signal in SEM. The production of back scattered electrons varies directly with the atomic number of the chemical elements present in the specimen, the higher the atomic number, the brighter that region will appear (*ana et al,. 2017*). SEM is a universal tool for morphological and size detection of silver nanoparticles.

UV-spectra recorded at different time intervals from arqueous solution of silver nitrate with *Gossypium arboreum* extract. The appearance of the peaks shows the characteristics of surface Plasmon resonance of silvernanoparticles. The UV- visible spectrum has an important role of silver nitrate and the presence of ingredients in the leaves for the formation of silver nano particles. The increase in the concentration of the leaf extract will also increase the absorption spectra of the silver nanoparticles formed

from aqueous extract of *Gossypium arboreum* leaves. The synthesized aqueous silvernanoparticles showed strong absorption band at 350nm for aqueous silver nanoparticles which was due to the surface Plasmon thus synthesized silver nanoparticles may be polydispersed, (*Anandlakshami et al., 2016*).

In the present investigation we have biosynthesized silver nanoparticles from *Gossypium arboreum* aqueous extracts because the extracts showed good antimicrobial activity. In our investigation we observed the silvernanoparticles synthesized using *Gossypium arboreum* extract showed a pronounced zone of inhibition, this is proved by the values of diameter of zone of inhibition obtained in table 1 and 2. There was significant zone of inhibition for silver nanoparticles synthesized from the plant extract, this indicate nanoparticles where having higher zone of inhibition. The antimicrobial activity of *Gossypium arboreum* leaves extracts and its synthesized silver nanoparticles were evaluated by disc diffusion method using bacteria and fungus which were found to have higher inhibitory action. The potential reason for the antimicrobial activity of silver is that AgNPs may attach to the surface of the cell membrane disturbing permeability and respiration functions of the cell. Smaller AgNPs having the large surface area available for interaction would give more antimicrobial effect than the larger AgNPs. It is also possible that silver nanoparticles not only interact with the surface of membrane, but can also penetrate inside the organism.

4.2 CONCLUSION

In the present study we have made an attempt to reduce the toxitcity caused by chemical used to reduce silver from silver nanoparticles by using the active constituents present in the plant extract of *Gossypium arboreum.* Nanoparticles biosynthesized by green synthesized in the present work were polydispersed and were characterized by different characterization techniques like UV-spectrophometer, phytochemical screening, scanning electron microscopy (SEM), fourier transform infrared spectroscopy (FTIR). We have characterized the shape, size, crystallinity and time required to synthesize nanoparticles. Our study demonstrated the biosynthesized silver nanoparticles were having good antimicrobial activity compared to the extracts of the plant.

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